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PRIMER NOTE

Polymorphic CA/GT and GA/CT microsatellite loci for *Ostrinia nubilalis* (Lepidoptera: Crambidae)

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Abstract

Ten polymorphic dinucleotide (CA/GT and GA/CT) microsatellite loci suitable for population genetic screening were characterized from enriched partial *Ostrinia nubilalis* genomic libraries. Sequence from 126 enriched small insert genomic library clones identified 25 CA/GT and 58 GA/CT loci that were unique. Perfect repeats tended to be short (n = 10–12). Ten microsatellites, PCR amplified from a Crawfordsville Iowa population showed a mean of 10 alleles per locus (range six to 20), and six of 10 loci showed heterozygote deficiency. Amplification of eight loci was observed in the sister species *O. furnicalis*.

Keywords: genetic marker, Lepidoptera, *Ostrinia nubilalis*

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Microsatellite markers are highly polymorphic and codominant, and useful for population genetic and genome mapping studies (Goldstein & Schlotterer 1999). Microsatellite isolation from Lepidoptera has been difficult (Nève & Meglècz 2000) and few reported from moths (Reddy *et al*. 1999; Ji *et al*. 2003).

Larval European corn borer, *Ostrinia nubilalis* (Hübner; Lepidoptera: Crambidae) infest *Zea mays* and cause major yield loss (Showers 1993). Coates & Hellmich (2003) showed geographical variation among *O. nubilalis* using two sex-linked markers, but acknowledged need from additional markers. *O. nubilalis* history and biology is of interest. First, *O. nubilalis* is native to Europe and western Asia, and genetic bottleneck and range expansion since North American introduction suggests a model for species introduction. Secondly, sympatric ecotypes differing in pheromone use, and generation number (voltinism) exist (Showers 1993). Co-existence and maintenance of distinct ecotypes suggests potential population and ecological studies.

Two dinucleotide microsatellite enriched partial *O. nubilalis* genomic libraries (CA/GT and GA/CT) were constructed from 12 individuals. Genomic DNA extracts were prepared using DNAeasy kits (Qiagen) and 1.25 μg digested in 50 μL reactions containing 10 U *Eco*RI (Promega), 10 U *Mse*I (New England BioLabs), 100 ng each *Mse*I and *Eco*RI adapter (Vos *et al*. 1995), 10 U T4 DNA ligase (Promega), 5 μL NEB buffer 2, and 5 μM ATP incubated 14 h at 37 °C. Ligations (250 ng) were mixed with 100 pmol 5′ biotin-TEG $(CA)_{12}$ or $(GA)_{12}$ probes in 100 μL 1X SSC, fragments denatured at 95 °C for 5 min, and hybridized at 55 °C for 5 min on a PTC-100 thermocycler (MJ Research). Streptavidin paramagnetic beads (100 μL; Promega) were added and incubated for 5 min, then washed twice with 800 μL 1X SSC at 55 °C for 5 min. DNA was released by incubation with 100 μL 80 °C deionized water for 5 min.

Recovered DNA fragments (10 μL) were polymerase chain reaction (PCR) amplified in 50 μL reactions with 5 μL 10X thermal polymerase buffer (Promega), 1.5 mM $MgCl_2$, 200 μM dNTPs, 3.5 U *Taq* polymerase (Promega), and 30 pmol *Eco*RI adapter primer (5′-GACTGCGTACCAATTC-3′) and *Mse*I adapter primer (5′-CGATGAGTCCC TGAG-TAA-3′). A PTC-100 thermocycler used 30 cycles of 96 °C for 20 s, 52 °C for 30 s, and 72 °C for 45 s, followed by 72 °C for 20 min. PCR product (1 μL) was ligated into 50 pmol pGEM-T easy cloning vector (Promega). Plasmids were cloned into *E. coli* SURE (Stratagene) by electroporation, recombinants identified by blue/white screening, isolated by alkaline lysis, and purified by PEG precipitation (Sambrook *et al*. 1989). Ten microliter (10 μL) DTCS Quickstart

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DNA sequencing reactions (Beckman-Coulter) used 1.6 pmol T7 primer. Sequence reaction products were purified by ethanol precipitation, suspended in 40 μL deionized formamide, and separated on a CEQ 8000 Genetic Analysis System (Beckman-Coulter) with method LFR-1 (denature: 90 °C for 120 s; inject: 2.0 kV for 15 s; and separated: 4.2 kV for 85 min in a 50 °C capillary).

Sequence from 126 plasmid inserts had 102 microsatellite loci, and 83 were unique; 25 CA/GT and 58 GA/CT microsatellites. Most repeats were short (n = 10–12 bp). PCR primer pair design was attempted for 44 cloned sequences containing perfect repeats using PRIMER 3 (Rozen & Skaletsky 1998). Acceptable PCR primers were not obtained from 14 sequences due to low GC content (< 40%), low T_m (< 55 °C), or self-annealing primer pairs. From 30 synthesized primer pairs (Integrated DNA Technologies) 10 PCR products were polymorphic, had ≤ 2 bands/individual, and were within predicted size ranges (Table 1).

Forward or reverse primer was synthesized and 5′-labelled with WellRed® dye (D2, D3, or D4; Proligo) for the 10 acceptable loci. PCR reactions contained 1.5 mM $MgCl_2$ (loci: OnGA15, OnGA32, OnCA14, OnCA46, and OnCA41) or 2.5 mM $MgCl_2$ (all remaining loci; Table 1), 150 μM dNTPs, 50 ng DNA, 2 pmol of each primer, 1 μL 10X thermal polymerase buffer (Promega), and 0.3125 U *Taq* DNA polymerase (Promega). A PTC-100 thermocycler used 96 °C for 3 min, then 35 cycles of 96 °C for 20 s, 30 s annealing (see Table 1), and 72 °C for 30 s. All touchdown (TD) PCR used stepdown from 67 °C at to 2 °C/cycle for 7 cycles, then 35 cycles with 52 °C annealing; other parameters identical to standard PCR. PCR product (0.5 μL) was added to 40 μL deionized formamide and separated on a CEQ 8000 Genetic Analysis System (Beckman-Coulter) using method Frag-3 (denature: 90 °C for 120 s; inject: 2.0 kV for 30 s; and separated: 6.0 kV for 35 min in a 50 °C capillary) with 0.25 μL 400 bp internal standard (Beckman-Coulter).

Allele number/locus ranged from six to 20 (mean 10 ± 3.944) among 61–91 *O. nubilalis* collected near Crawfordsville, Iowa (Table 1). Expected heterozygosity (H_E) was calculated according to Nei (1973) and differences with observed heterozygosity (H_O) tested by chi-square (χ^2). Calculations used POPGENE software version 1.32 (Table 1; Yeh & Boyle 1999). Four of 10 *O. nubilalis* microsatellite loci were in Hardy–Weinberg equilibrium (HWE) and the remainder (60%) showed heterozygote deficiencies. High *O. nubilalis* homozygosity may be due to genetic bottleneck at North American introduction, range expansion, or local inbreeding (Hartl & Clark 1997). ARLEQUIN software detected no linkage disequilibrium between loci (Schneider *et al*. 1997; results not shown).

Table 1 Ten *Ostrinia nubilalis* CA/GT and GA/CT microsatellite markers. Primers, repeat unit, GenBank accession, annealing temperature (T_m), PCR product size range, allele number, and sample number are given for each locus. The 5′-labelled primers are indicated with appropriate WellRed® dye (D2, D3, or D4; Proligo). The observed heterozygosity (H_O) was obtained from a Crawfordsville, Iowa population sample and expected heterozygosity (H_E) calculated according to Nei (1973). # indicates significant difference between H_E and H_O. *Ostrinia furnicalis* (*O. furn*.) amplification success using *O. nubilalis* PCR reaction parameters (see methods) was judged from four samples

Locus	Primer sequence	Repeat	GenBank accession	T_m (°C)	PCR size range (bp)	Allele No.	Sample No.	H_O	H_E	*O. furn.* PCR ±
OnGA16	F-D2-CTGCGTACATGCAGCGTAAA R-TCATCACCATCGATGAGTCC	$(GA)_{12}$	AY642964	TD	124–134	6	75	0.393	0.643 #	+
OnGA28	F-D4-CTGCGTACATGCAGAATAAGGG R-TTTTATCAAACTCTTA*YR*CGATGAAGC	$(GA)_{11}$	AY642965	52	86–104	8	87	0.368	0.742 #	+
OnGA32	F-D4-GGCCTGATCATTTCCCTGAG R-TGCTGCATGTACGCAGTCAG	$(CT)_{15}$	AY642967	55	150–166	8	89	0.461	0.487	+
OnGA33	F-D3-TCAAACTCTTATGCGATGAAGC R-CTGCAGAATAAGGGGCACT	$(GA)_{13}$	AY642968	59	73–105	13	91	0.528	0.637	+
OnGA39	F-D3-TCTCAAGCACTTTGATCCGG R-GGCTGAAGTTGGAAGAGGATCT	$(CT)_8$	AY642969	TD	88–104	9	86	0.198	0.606 #	+
OnCA07	F-D2-GAATTCATTTTACTCAAGATTTGTTTA R-TAATATGGCCTGATGGTGTGCA	$(CA)_{11}$	AY642970	55	115–133	8	67	0.210	0.755 #	–
OnCA16	F- D4-CCTGACTTGTGCCGAGTAGGT R-AACATGGCCGCAATATAGGC	$(CA)_9$	AY642971	TD	127–145	10	61	0.613	0.727	+
OnCA27	F-D2-CCTGACTTGTGCCGAGTAGGT R-CAACATGGCCGCAATATAGGC	$(CA)_{18}$	AY642972	TD	126–146	9	89	0.562	0.626	+
OnCA41	F-D2-GCCTAAGGCAGGAATTGAACC R-CGGAAACCTTACTCCAAACTCTC	$(CA)_{14}$	AY642973	TD	189–247	20	86	0.439	0.855 #	–
OnCA46	F-D2-GTAGGGCTACCCATCACGCACA R-CAAAAGTAAGGTAAGGCTATGTGAC	$(CA)_{10}$	AY642974	57	67–83	9	88	0.455	0.733 #	+

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References

Coates BS, Hellmich RL (2003) Two sex-linked microsatellite loci show geographic variance among North American *Ostrinia nubilalis*. *Journal of Insect Science*, **2**, 29.

Goldstein DB, Schlotterer C (1999) *Microsatellites: evolution and applications*. Oxford University Press, Oxford, UK.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*. Sinauer, Sunderland, MA.

Ji YJ, Zheng DX, Hewitt GM, Kang L, Li DM (2003) Polymorphic microsatellite loci for the cotton bollworm *Helicoverpa armigera* (Lepidoptera: Noctuidae) and some remarks on their isolation. *Molecular Ecology Notes*, **3**, 102–104.

Nei M (1973) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Nève G, Meglècz M (2000) Microsatellite frequencies in different taxa. *Trend in Ecology and Evolution*, **15**, 376–377.

Reddy KD, Abraham EG, Nagaraju J (1999) Microsatellite in the silkworm, Bombyx mori: abundance, polymorphism, and strain characterization. *Genome*, **42**, 1057–1065.

Rozen S, Skaletsky HJ (1998) PRIMER 3. Available from http://genome.wi.mit.edu/genome_software/other/primer3.html

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring. Harbor Laboratory Press, Cold Spring Harbor, NY.

Schneider S, Kueffer JM, Roessli D, Excoffier L (1997) ARLEQUIN ver. 1.1: a software for population genetic data analysis. http://lgb.unige.ch/arlequin/.

Showers WB (1993) Diversity and variation of European corn borer populations, In: *Evolution of Insect Pests/Patterns of Variation* (eds Kim KC, McPherson BA) pp. 287–309. Wiley and Sons Inc, New York, NY.

Vos P, Hogers R, Bleeker M *et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Yeh CF, Boyle T (1999) POPGENE Version 1.32: Microsoft Window-based freeware for population genetic analysis. http://www. ualberta.ca/~fyeh/.